SOIL ALGAE OF FORTY PONDS UNDER CONSTRUCTION AT ITHACA, NEW YORK[1]

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To allow experimental study of basic ecology of aquatic weeds and their control by herbicides, 92 ponds have been constructed at two locations in the Ithaca area. The purpose of this study was to determine the algal flora of the soil from which one group of 40 ponds was constructed. Such algae represent a major source of introduction for the algal populations that are expected to appear in the ponds when they are completed and filled.

Construction of the set of ponds used in this study was begun in July, 1962 and completed in November, 1963. Each pond is 66 feet square in the bottom dimension and 124 feet square in the top dimension when measured from center dike to center dike. The area covered by one pond is one-third of an acre at the top and one-tenth of an acre at the bottom. Proposed depths of the ponds when filled range from five to eight feet. The ponds were constructed from abandoned farm lands. The soil, Volusia silt loam, had not been recently disturbed when construction was begun.

The group of ponds under study is arranged in two rows of ten ponds each on each side of a supply canal; a second canal runs perpendicular to the first canal at the end region of the ponds (Fig. 1).

Alternate ponds and alternate banks of ponds with respect to the supply canal were sampled just after they had been given final shape. Three samples were taken from the soil at each pond: the top sample was obtained from the upper margin of the pond, the middle sample from approximately three to five feet down the slope of the pond, and the bottom sample from the floor of the pond. In each case, the top three

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Plate 1313

Figure 1. Ponds at time of sampling.

to six inches of soil was sampled in a way that yielded well-mixed 10 gm samples. Twenty ponds were sampled initially, 25 in all. Half of the initial samples were examined immediately and the other half refrigerated. Because the latter froze accidentally, new samples were collected two months later from the same locations.

Five media were used in culturing the samples: Modified Bristol's Solution (Bold, 1949) with trace elements (Chantanachat and Bold, 1962); Knop's Solution (Bold, 1942); Kratz and Myer's (1955) Medium D; Modified Detmer's Solution (Bold, 1942); Soil Extract Solution (Bold, 1942).

Culture vessels were 50 ml Delong culture flasks and 18 × 150 mm test tubes. Both flasks and tubes were capped with stainless steel closures (Bellco). Flasks contained 20 ml of media. Inocula consisted of one gram portions of soil.

Original isolations and subsequent transfers were placed in a culture room under standard conditions of twenty-four hour illumination at 250-450 foot candles (warm white fluorescent) on one shelf and 575-675 foot candles on another and temperature of 20°C. ± 2°C.

To facilitate identification, various techniques were employed for separating the original populations into subcultures containing fewer species each. By these procedures, debris was removed and cultures were obtained in which one or few algal species were isolated. No attempt was made to produce cultures free of bacteria.

Flasks were checked daily for presence of algal growth. Typically, the meniscus showed green color within one to two weeks. Flasks were subcultured by the methods of Bold (1942), Pringsheim (1946; 1950), Deason and Bold (1960), Chantanachat and Bold (1962), and Mattox and Bold (1962). These methods include micromanipulation with drawn-out Pasteur capillary pipettes, streaking on agar, and inoculation into fresh media. Transfers, using the above methods, were made into Bristol's liquid medium and on the same solidified with 1.5 per cent agar; into the original medium; and into soil water tubes (Pringsheim, 1950) made with tap water and sterilized by repeated steaming.

A method used by Warcup (1950) for isolating soil fungi was also employed for isolation of soil algae. This method involves distribution of soil particles throughout a thin layer of solid nutrient medium. Small amounts of soil were used as inocula directly and in dilutions (with water) of 1:25, 1:125 and 1:625.

A method involving dispersal of soil particles through liquid nutrient medium, proposed by Willson and Forest (1957), was also employed.

Transfers were made periodically to keep the algae in the best possible condition. Colonies from petri dishes were transferred to Bristol's liquid and 1.5 per cent agar media. Isolations were made from flasks and Willson-Forest Petri dishes by means of capillary pipettes or glass needles.

Fresh mounts in distilled water were used predominantly in identification of the algae. India ink and iodine were used as necessary. Aceto-carmine preparations (Deason and Bold, 1960) proved less satisfactory than the freshmount method.

Algae were identified to species using standard taxonomic references. For the majority of identifications, G. W. Prescott's *Algae of the Western Great Lakes Area* (1962) served

as the primary source. Geitler's *Cyanophyceae* (1932) and Collins' *Green Algae of North America* (1928) were also used in particular instances. In addition, University of Texas *Phycological Studies*, I-III (Deason and Bold, 1960; Chantanachat and Bold, 1962; Mattox and Bold, 1962) proved useful because of their parallel nature.

Identification of species of *Bacillariophyceae* and certain *Chlorococcales* was not attempted; nevertheless, an attempt was made to estimate their numbers. Patrick's key to genera of *Bacillariophyceae* (1959) was helpful in this connection, and the following technique was used to aid in estimating the number of *Chlorococcales*.

Six flasks were chosen at random from a group of fifty flasks containing putative *Chlorococcales*, as determined by microscopic examination. Five successive 1 to 5 dilutions were made in sterile water from cells thrown down by centrifuging the original culture. These were plated out on 1.5 percent Bristol's agar in twenty-five Petri dishes. When growth was evident, fifty well-separated colonies were selected at random from the twenty-five Petri dishes and inoculated into Bristol's liquid medium. The tubes were returned to the culture room and examined approximately three to four weeks later when good growth was apparent.

Criteria used for determining the presence of differing entities among these isolates included presence or absence of green meniscus in test tube; presence and amount of sludge; presence or absence of flagellated entities in microscopic examination; and morphology of adult cells, including thickness of cell wall, number of pyrenoids, and shapes of chloroplasts (Deason and Bold, 1960; Chantanachat and Bold, 1962; Mattox and Bold, 1962; Herndon, 1958; and Starr, 1955).

RESULTS

Seventy-two species of algae have been identified from the soil samples. Divisions represented include *Cyanophyta* - 43 species, *Chlorophyta* - 29 species, and *Chrysophyta (Bacillariophyceae)*, species not identified. A taxonomic list of species is presented in Table I.

Data were examined for possible correlations of differences among the species of algae found in the top, middle, and lower levels of the pond walls and among their distributions horizontally from pond to pond. Vertical distribution of representative algae appearing frequently, relatively frequently, and rarely is given in Table II. Considerable range in frequency of occurrence was found in both distributions, but less definite patterns were noted in the horizontal distribution.

Among the *Chlorococcales* two members were identified as *Chlorococcum* and *Chlorella*, and two of the latter were keyed out further to species. Other genera and species could not be ascertained with security with the criteria available.

Members of the *Chlorococcales*, particularly species of *Chlorococcum*, appeared in every soil sample analyzed. Diatoms appeared in thirty-eight out of forty-five samples inoculated into the Delong culture flasks.

Of particular interest were four species of algae described from Texas soils (Deason and Bold, 1960; Chantanachat and Bold, 1962; Mattox and Bold, 1962): *Hormidium flaccidum*

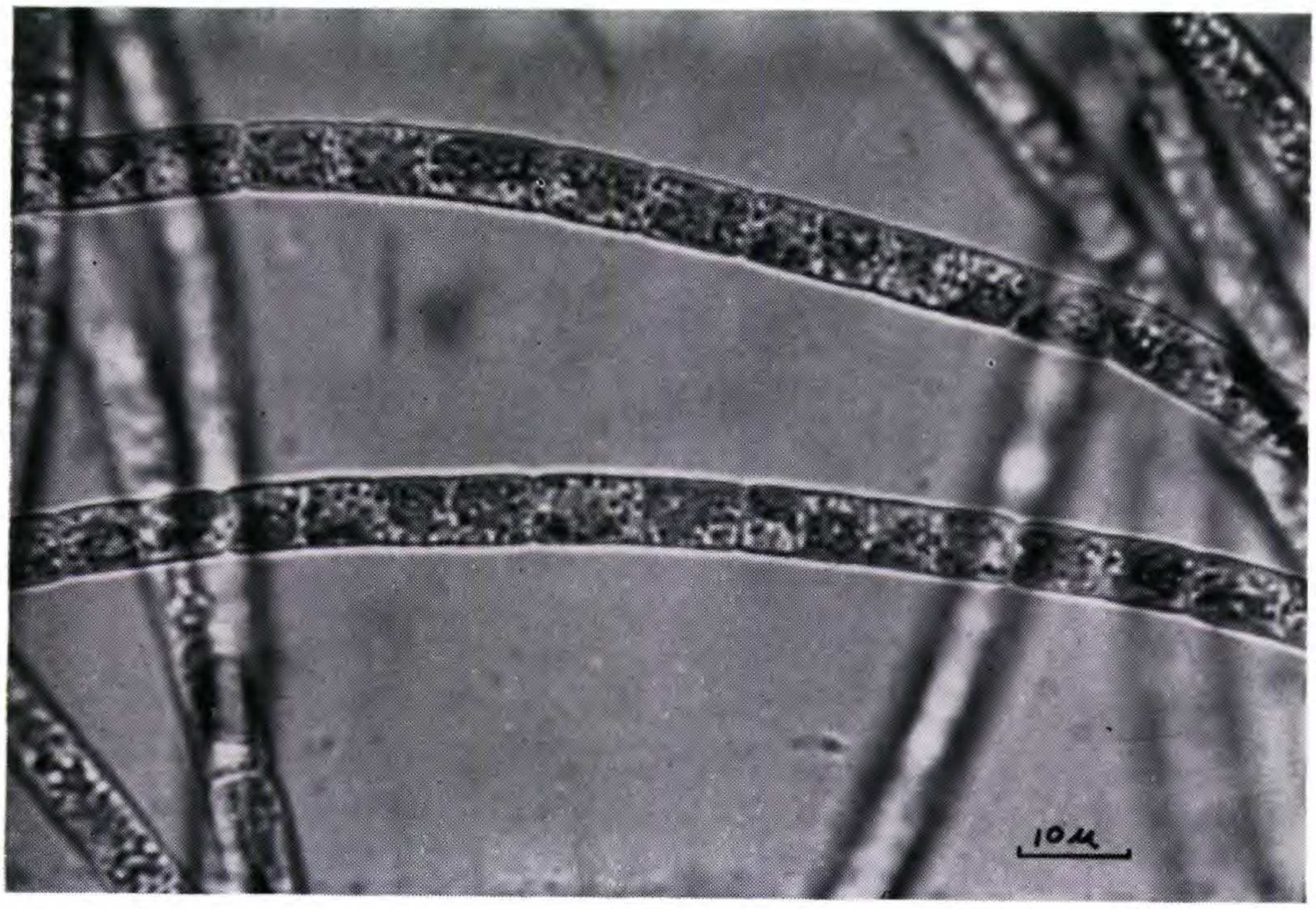

Plate 1314

Figure 2. *Hormidium flaccidum*. One-month-old filaments.

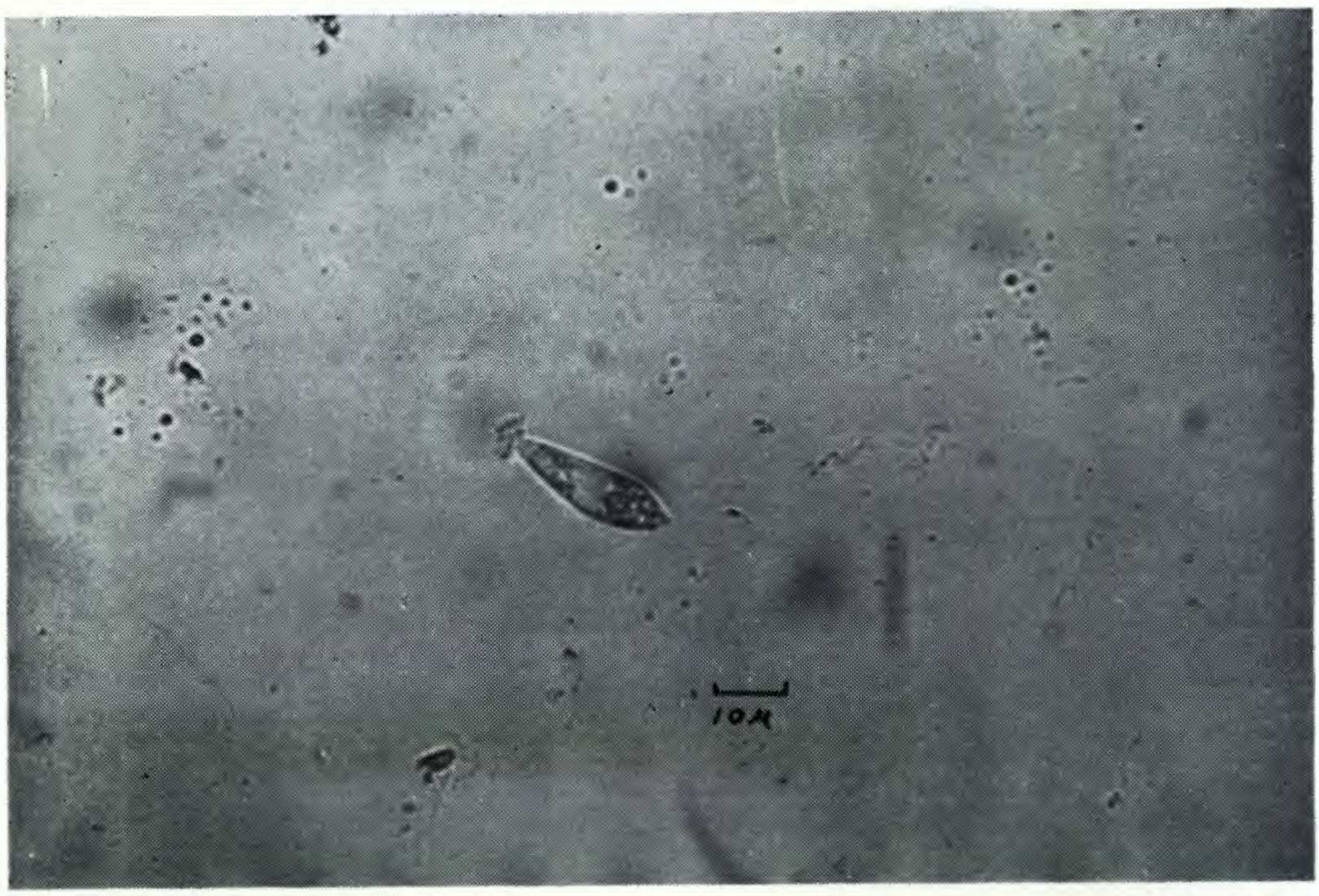

Plate 1315

Figure 3. *Ulothrix belkae*. Germling.

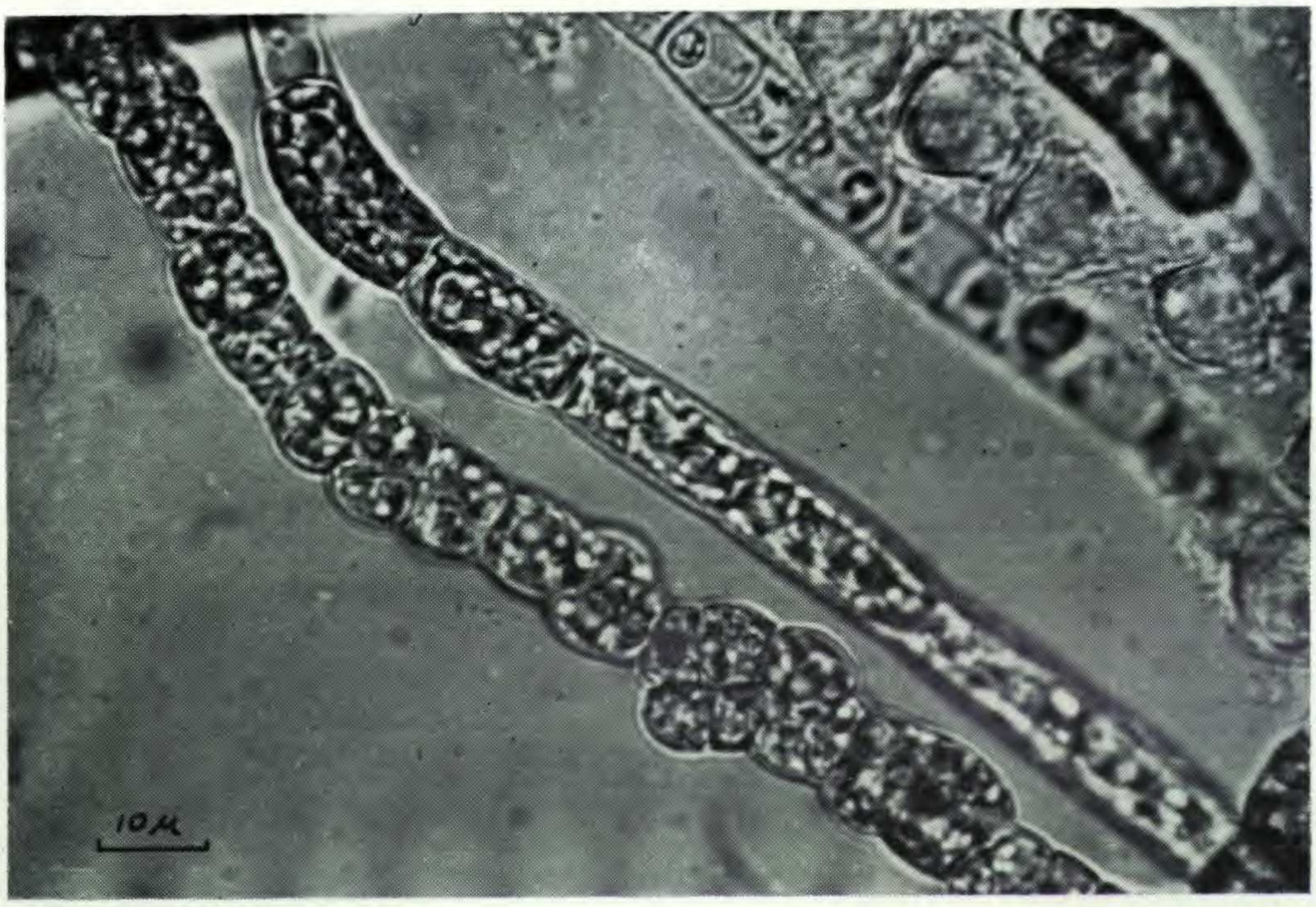

Plate 1316

Figure 4. *Ulothrix belkae*. Two-month-old filaments.

(Fig. 2), *Ulothrix belkae* (Fig. 3, 4), *Chlamydomonas aggregata*, and *Chlamydomonas typica*.

Chlamydomonas appeared frequently in the samples in both vertical and horizontal distributions but could not always be keyed out to species.

One species of *Chlorosarcinopsis* was recovered from the soil samples. On the basis of descriptions by Herndon (1958), it was identified as *Chlorosarcinopsis eremi*.

DISCUSSION

The five media used in this experiment proved to be selective to some degree. Bristol's solution was least selective, and for the most part, the algae grew well in this medium. For this reason, Bristol's solution was used predominatly as the medium for transfer. Knop's solution appeared selective for certain green algae, specifically *Chlorococcales*, although some blue-green algae were also isolated from this medium. Kratz and Myer's medium yielded an abundance of blue-green algae, with a large number of *Chlorococcales* also. In general, the soil-extract solution produced poor growth and appeared to support only a few diatoms, *Chlorococcales*, and blue-greens, the latter being mostly in the state of fragmented filaments. Detmer's medium appeared to select for diatoms and blue-greens.

Members of the Division *Cyanophyta* predominated in the samples. Of seventy-two species identified, forty-three species were blue-green algae. Thirty-five of the species of *Cyanophyta* were filamentous in structure; seven were coccoid (unicellular or colonial); and one was a species of *Spirulina*. Of the filamentous blue-greens, twelve were species of *Oscillatoria*, and six each were species of *Anabaena* and *Nostoc*. Other genera were represented by fewer species.

Blue-green algae grew well in Kratz and Myer's Medium D, pH 8.0. The pH of the soil samples also was slightly basic (7.4).

Diatoms were found in high frequency in the soil samples which also contained a large number of blue-green algae. These results differ from those of Bristol (1920) who re-

ported that soils rich in blue-green algae usually contained only few species of diatoms, and vice-versa.

The large amount of *Chlorococcum* reported in this study agrees with observations of Smith (1944) who likewise found an abundance of *Chlorococcum (humicolum)* in four different soil types analyzed in a study of soils from Florida.

Another genus frequently encountered in this study was *Chlamydomonas*. Lund (1947) also reports the common occurrence of species of *Chlamydomonas* in soil, presenting ten new species in his survey. The taxonomy of *Chlamydomonas* is complicated by the very large number of described species and the frequent appearance of new species descriptions.

Isolation methods were not equally successful. Isolation by streaking out on 1.5 percent Bristol's agar and by means of micromanipulation with drawn-out Pasteur capillary pipettes proved to be most often satisfactory. The Willson-Forest plating method proved excellent for the isolation and growth of blue-greens. An outstanding advantage of this method is the fact that filamentous forms could be transferred from dried-out plates and, in fresh media, growth was generally good. The modified serial-dilution and plating technique was satisfactory. Well-isolated colonies on the Petri dishes frequently yielded uni-algal cultures. The Warcup method, although it eliminated the need for such serial-dilution techniques as those proposed by Bold (1942), Skinner (1932), and Bristol-Roach (1962), is limited to the use of extremely small inocula, and the plates are prone to rapid contamination with bacteria and fungi.

Several conclusions may be drawn regarding the frequency of occurrence and distribution of species among the samples taken at different levels from the ponds. In general, most species were found relatively frequently and were equally distributed in the three levels. More than 75 percent of the species were found in all three levels. However, somewhat fewer isolations could be obtained from lower levels than from higher. One hundred seventy-nine species isolations were obtained from soil samples taken at the top level;

160 at the middle level; and 141 at the lower level. Some exceptions to the generality that most species were found in all three levels are noted in Table II. It shows that some species occurred with equal frequency in the top two levels but with lower frequency in the bottom level. On the other hand, some species appeared with equal frequency in the top two levels yet at a higher frequency in the bottom level. Few species occurred exclusively in the top, middle, or lower level.

Because the ponds were still under construction at the time of sampling and were subject to frequent mechanical disturbance, it is assumed that the populations were not well established. However, some differences in habitat among the three levels were clear. The lower level of the ponds was subject to more mixing and also to moistening with rain water which remained in the new pond bottoms for long periods of time. This alone could account for the difference in frequency of occurrence of species as compared to the top two levels. Reasons for the appearance of a few species one or few times may be that occasional air-borne forms were involved, that the media were not always inducive to good growth of certain species, or that occasional resistant forms became vegetative.

The ability to persist in dormant condition under periods inimical to growth was probably a factor in determining the types of algae found and the period in culture before they appeared. Growth of members of the *Chlorophyta* was rapid, with visible green color appearing in one to two weeks. Likewise, when transfers to fresh media were made, zoosporulation usually occurred within a few days to a week. Certain species, however, did not appear for several weeks to several months, probably because they required the prior germination of resistant structures. Blue-green algae, in general, appeared later than the majority of green algae but were present in most samples eventually and remained viable longer.

The samples that were accidentally frozen while being stored were used in the Warcup and Willson-Forest isolation

techniques. In general, blue-greens were the predominant forms isolated. The species of blue-greens which were isolated from the frozen samples compared closely with those recovered from non-frozen samples.

Smith (1951) reported that the bulk of species of soil algae are generally made up of *Myxophyceae* (blue-green algae) and *Bacillariophyceae*, with *Chlorophyceae* third, and *Xanthophyceae* and *Euglenophyceae* contributing a much smaller number. Blue-green algae predominated in this study, but members of the *Chlorophyceae* and *Bacillariophyceae* appeared with almost equal frequency, while no species of *Xanthophyceae* or *Euglenophyceae* were found, perhaps because the media did not select for them.

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TABLE I

TAXONOMIC LIST OF ALGAE IDENTIFIED

CHLOROPHYTA

CHLOROCOCCALES
CHARACIACEAE
Characium obtusum A. Braun
CHLOROCOCCACEAE
Chlorococcum spp.
COELASTRACEAE
Coelastrum microporum Naegeli
OOCYSTACEAE
Chlorella ellipsoidea Gerneck
Chlorella vulgaris Beyerinck
Oocystis crassa Wittrock
Oocystis parva West and West
Trochiscia reticularis (Reinsch) Hansgirg
PROTOSIPHONACEAE
Protosiphon botryoides (Kützing) Klebs
SCENEDESMACEAE
Gloeocystis vesiculosa Naegeli
Palmella mucosa Kützing
ULOTRICHALES
ULOTRICHACEAE
Hormidium flaccidum A. Braun
Stichococcus bacillaris Naegeli
Ulothrix belkae Mattox and Bold
Ulothrix subtilissima Rabenhorst
MICROSPORACEAE
Microspora stagnorum (Kützing) Lagerheim
CHAETOPHORACEAE
Protoderma viride Kützing
ULVALES
SCHIZOMERIDACEAE
Chlorosarcinopsis eremi Herndon
VOLVOCALES
CHLAMYDOMONADACEAE

Scenedesmus hystrix Lagerheim
Scenedesmus obliquus (Turpin) Kützing
Scenedesmus quadricauda (Turpin) de Brebisson
TETRASPORALES
PALMELLACEAE
Gloeocystis ampla (Kützing) Lagerheim
Gloeocystis gigas (Kützing) Lagerheim
Chlamydomonas aggregata Deason and Bold
Chlamydomonas snowii Printz
Chlamydomonas typica Deason and Bold
Sphaerellopsis fluviatilis Pascher
VOLVOCACEAE
Gonium pectorale Mueller
ZYGNEMATALES
DESMIDIACEAE
Cosmarium spp.

CYANOPHYTA

CHROOCOCCALES
CHROOCOCCACEAE
Aphanocapsa elachista West and West
Chroococcus minor (Kützing) Naegeli
Dactylococcopsis smithii Chodat and Chodat
Synechococcus aeruginosa Naegeli
Synechocystis aquatilis Sauvageau
Synechocystis crassa Woronichin
Synechocystis sallensis Skuja
HORMOGONALES
NOSTOCACEAE
Anabaena affinis Lemmermann
Anabaena cylindrica Lemmermann
Anabaena flos-aquae (Lyngbye) de Brebisson
Anabaena oscillaroides Bory
Anabaena subcylindrica Borge
Anabaena torulosa (Carmichael) Lagerheim
Anabaenopsis elenkinii Miller
Cylindrospermum catenatum Ralfs
Cylindrospermum marchicum Lemmermann
Nodularia harveyana Thuret
Nodularia spumigena Mertens
Nostoc commune Vaucher
Nostoc linckia (Roth) Bornet and Thuret
Nostoc muscorum C. A. Agardh
Nostoc paludosum Kützing
Nostoc sphaericum Vaucher
Nostoc verrucosum Vaucher
OSCILLATORIACEAE
Lyngbya limnetica Lemmermann
Lyngbya martensiana Meneghini
Lyngbya versicolor (Wartmann) Gomont
Oscillatoria acutissima Kufferath
Oscillatoria agardhii Gomont
Oscillatoria amoena (Kützing) Gomont
Oscillatoria amphibia C. A. Agardh
Oscillatoria angusta Koppe
Oscillatoria angustissima West and West
Oscillatoria limnetica Lemmermann
Oscillatoria rubescens De Candolle
Oscillatoria splendida Greville
Oscillatoria subbrevis Schmidle
Oscillatoria tenuis C. A. Agardh
Oscillatoria terebriformis C. A. Agardh
Phormidium minnesotense (Tilden) Drouet
Schizothrix calcicola (Agardh) Gomont
Spirulina major Kützing
RIVULARIACEAE
Calothrix stagnalis Gomont

TABLE II

Vertical distribution of representative algae found frequently, less frequently, and rarely in soil samples.

FREQUENCY OF OCCURRENCE	NUMBER OF OCCURRENCES		
	TOP	MIDDLE	LOWER
FREQUENT			
Oscillatoria limnetica	13	13	13
Synechocystis aquatilis	14	14	13
Chlamydomonas spp.	11	9	6
Oscillatoria angusta	8	8	12
LESS FREQUENT			
Scenedesmus quadricauda	5	4	2
Anabaena cylindrica	4	4	5
Nostoc commune	4	3	4
Oscillatoria agardhii	2	2	6
RARE			
Spirulina major	0	0	1
Anabaena oscillaroides	0	0	1
Phormidium minnesotense	1	0	0
Anabaenopsis elenkinii	1	0	0
Oscillatoria splendida	0	1	0
Anabaena flos-aquae	0	1	0

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